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A Novel Antiinflammatory Role for the Short-Chain Fatty Acids in Human Labor

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Human parturition is an inflammatory process that can be activated prematurely by pathological stimuli. This study investigated the expression of G protein-coupled receptors GPR43 and GPR41 receptors in human uteroplacental tissues and the role of short-chain fatty acids (SCFA) in modulating inflammatory pathways in fetal membranes. Expression of GPR43 and GPR41 was investigated in uteroplacental tissues collected from women delivering at term or preterm after ethical approval and patient informed consent. The effect of SCFA on expression of inflammatory genes was assessed in amnion explants after culture with a mimetic of infection (lipopolysaccharide, LPS). Sodium propionate effect on LPS-induced neutrophil chemotaxis was evaluated by transwell assay. GPR43 and GPR41 mRNA expression was higher in myometrium and fetal membranes collected from women after the onset of labor. GPR43 protein expression localized to immune cells and vascular endothelium in the myometrium and epithelium of fetal membranes. Treatment with LPS significantly increased mRNA expression of GPR43 and inflammatory genes. Cotreatment with LPS and sodium propionate decreased LPS-induced expression of inflammatory genes including IL-6, IL-8, cyclooxygenase-2, IL-1 α , intercellular adhesion molecule-1, and platelet endothelial cell adhesion molecule-1 but not IL-1 β or lymphocyte function-associated antigen-1. Sodium propionate reduced LPS-induced neutrophil chemotaxis and protein secretion of the neutrophil chemoattractant IL-8. Finally, fetal membrane expression of GPR43 was significantly higher in women delivering preterm with evidence of infection. GPR43-SCFA interactions may represent novel pathways that regulate inflammatory processes involved in human labor. Suppression of inflammatory pathways by SCFA may be therapeutically beneficial for pregnant women at risk of pathogen-induced preterm delivery. (*Endocrinology* 153: 395–403, 2012)

There is increasing evidence that human parturition is associated with activation of inflammatory processes in the uteroplacental unit (1, 2). These include a dramatic influx of immune cells, increased expression and secretion of inflammatory cytokines and chemokines (3, 4), and tissue remodeling facilitated by production of matrix metalloproteinases (5, 6). Additionally, in labor, peripheral blood leukocytes have an increased chemotactic ability, suggesting that inflammation is not just confined to

gestational tissues (7). Pathological stimulation of inflammatory events can lead to preterm labor (8), a major obstetrical complication. Nevertheless, the mechanisms regulating the inflammatory processes of parturition and its premature activation remain incompletely characterized.

In this study, we examined a putative role for short-chain fatty acid (SCFA) signaling through the G protein-coupled receptors (GPR43) and GPR41. These receptors have been reported to bind to SCFAs such as acetate, pro-

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Abbreviations: CA, Chorioamnionitis; COX, cyclooxygenase; GPR43, G protein-coupled receptor; ICAM, intercellular adhesion molecule; LPS, lipopolysaccharide; PECAM, platelet endothelial cell adhesion molecule; pPROM, preterm premature rupture of membranes; PTL, preterm labor; qRT-PCR, quantitative real-time PCR; SCFA, short-chain fatty acid.

pionate and butyrate (9, 10). Acetate and propionate have been shown to be the most specific and potent ligands for GPR43, whereas propionate and butyrate can equally bind GPR41 and GPR43 (9, 11). Although sharing the same ligands, SCFA receptors show different tissue distribution profiles. GPR43 is highly expressed in hematopoietic tissue (spleen, bone marrow) and immune cells, in particular neutrophils and monocytes, whereas GPR41 is more widely expressed (12).

SCFAs are products of the anaerobic bacterial fermentation of unabsorbed carbohydrates in the human intestine, thus representing the fermentable dietary fiber. They are subsequently transferred to the bloodstream after absorption by the intestinal mucosa. SCFA concentrations increase with inflammatory disease, and high levels are commonly found at sites of anaerobic bacterial infection (13, 14). There is emerging evidence on the effects of SCFAs in other tissues and organs (9, 15), including involvement in immune processes, mostly as antiinflammatory agents. They can regulate neutrophil function (16) and modulate inflammatory cytokine expression, especially in the presence of inflammatory stimuli (12, 17, 18). The highly selective expression of GPR43 on immune cells and the parallel functional effects of SCFA on neutrophils (9, 19) suggest that GPR43 receptor may specifically mediate SCFA inflammatory effects.

This study investigated the following: 1) GPR43 and GPR41 mRNA expression in human gestational tissues at term and their regulation by lipopolysaccharide (LPS), 2) the *in vitro* effect of SCFA on LPS-induced expression of proinflammatory cytokines and adhesion molecules in human amnion explants, and 3) GPR43 and GPR41 mRNA expression in gestational tissue from women affected by spontaneous preterm labor (PTL) and preterm premature rupture of membranes (pPROM) with or without chorioamnionitis (CA).

Materials and Methods

Patients and tissue collection

Uteroplacental tissues (myometrium, placenta, amnion, chorion) were collected from a total of 22 women at term of gestation (>37 wk of gestation) delivering by cesarean section, of whom 11 were nonlaboring (group A) and 11 were in labor of spontaneous onset (group B) (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at <http://endo.endojournals.org>). From all term pregnant patients, samples of myometrium, placenta, amnion, and chorion were collected and, from nonlaboring women, a blood sample was taken for neutrophil chemotaxis assay (described in a separate section). Nonlaboring women underwent elective cesarean section before the onset of labor for indications such as breech presentation and previous cesarean section, whereas la-

boring women underwent cesarean section for indications such as cardiocotographic abnormalities and failure to progress in the first stage of labor. All were healthy women with singleton pregnancy receiving perinatal care at the Simpson Centre for Reproductive Health, The Royal Infirmary of Edinburgh, UK. Women with medical complications such as hypertensive disorders, diabetes, pyrexia, and infections and those taking medication including steroids and prostaglandins were excluded from the study. All tissues were collected and immediately either placed in RNAlater (Applied Biosystems, Warrington, UK) and frozen at -80°C until assay or fixed in 4% neutral buffered formalin and wax embedded for immunohistochemistry or placed in RPMI 1640 (Sigma, Poole, UK) (containing 2 mmol L-glutamine, 100 IU penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin) and transported to the laboratory for *in vitro* experiments.

A second cohort of samples (placenta and fetal membranes) was collected from 26 women admitted to the Division of Obstetrics and Gynecology, Policlinico Santa Maria alle Scotte, Siena, Italy, and who delivered preterm (Supplemental Table 2). The women had regular uterine contractions occurring at a frequency of at least two to three every 10 min associated with cervical changes between 23 and 34 wk of gestation. All patients received 12 mg dexamethasone administered im twice with an interval of 12 h to induce fetal lung maturity, and all of them delivered within 24 h after dexamethasone treatment. All women with pPROM received antibiotic therapy. Women delivering preterm were divided into three groups according to the following diagnoses: 1) PTL with intact membranes without histological chorioamnionitis ($n = 9$); 2) pPROM, defined as spontaneous rupture of the membranes within 37 wk gestation at least 1 h before the onset of contractions ($n = 10$); and 3) pPROM with histologic CA diagnosed by the presence of inflammatory cells in the chorionic plate and/or chorioamniotic membranes ($n = 7$). Women with cervical incompetence, uterine malformations, polyhydramnios, multiple gestation, and fetal-maternal complications (thyroid disease, asthma, cardiovascular diseases, diabetes, hypertension, preeclampsia, abruptio placentae, fetal growth retardation and fetal malformation) were excluded from the study. The gestational age was assigned either on the basis of the last menstrual period or ultrasound before 20 wk of gestation. Shortly after collection, tissues were placed in RNAlater (QIAGEN, Milan, Italy) and frozen at -80°C until assay.

Ethical approvals were obtained from the respective local research ethics committees in Edinburgh and Siena. Written informed consent was obtained from all subjects before tissue collection.

Amnion explants and treatment

Amnion from women in group A was transported in RPMI 1640, extensively washed in PBS, dissected into 8-mm discs of tissue using a cork borer, and incubated overnight at 37°C in serum-free RPMI 1640 medium. The explants were then placed in 24-well plastic plates, with two discs of tissue per well. The wells contained 1 ml RPMI 1640 as a vehicle, 100 ng/ml LPS, 100 ng/ml LPS and 20 mmol sodium acetate, or 100 ng/ml LPS and 20 mmol sodium propionate and incubated at 37°C for 8 or 24 h (12, 20). Subsequently, supernatant and tissue were snap frozen on dry ice and stored at -20°C and -80°C , respectively.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from tissues using the QIAGEN RNeasy minikit (QIAGEN Ltd., Crawley, West Sussex, UK), according to the manufacturer's instructions. RNA concentration (nanograms per microliter) for each sample was measured using a NanoDrop spectrophotometer ND-1000 (Thermo Fisher Scientific Inc., Wilmington, DE). cDNA was synthesized using a SuperScript VILO cDNA synthesis kit (no. 11754-250; Invitrogen Ltd., Paisley, UK). qRT-PCR was performed for SCFA receptors (GPR43, GPR41), proinflammatory cytokines [IL-1 α , IL-1 β , IL-6, IL-8, cyclooxygenase (COX)-2] and adhesion molecules [intercellular adhesion molecule (ICAM)-1, lymphocyte function-associated antigen-1 (LFA-1), platelet endothelial cell adhesion molecule (PECAM)-1] using Express qPCR Super-Mix (no. 11785-200; Invitrogen). The sequences of primers and probes for genes used for qRT-PCR are shown in Supplemental Table 3. The qRT-PCR were carried out using an ABI 7900HT Fast real-time PCR system machine (Applied Biosystems). Gene expression was normalized for RNA loading using 18S rRNA as internal standard and results are presented relative to a positive RNA standard (cDNA obtained from endometrial adenocarcinoma) included in all reactions.

Immunohistochemistry

Tissue sections (5 μ m) were dewaxed and rehydrated, and antigen retrieval was performed by treating sections for 5 min in a pressure cooker in boiling 0.01 M citrate buffer (pH 6.0). Twenty-four sections were analyzed from four women. Endogenous peroxidase activity was quenched with 10% (vol/vol) H₂O₂ in methanol for 30 min at room temperature. Immunohistochemistry for GPR43 was performed using an automated Visions Biosystems bond immunohistochemistry system (Leica Microsystems Ltd., Milton Keynes, Bucks, UK). Sections were incubated for 120 min using a rabbit antihuman polyclonal antibody to GPR43 (LS-A6598; LifeSpan Biosciences Inc., Seattle, WA) at a dilution of 1:75 or omission of the primary antibody as a negative control. Sections were subsequently treated for 15 min with polymer linked antirabbit peroxidase and counterstained in hematoxylin and mounted in Pertex (Cellpath PLC, Powys, UK). Images were captured using an Olympus Provis AX70 microscope (Olympus U.K. Ltd., Essex, UK) equipped with an AxioCam HRc camera (Carl Zeiss AG, Jena, Germany) and AxioVision 4.8 Imaging Software (Carl Zeiss).

Neutrophil chemotaxis assay

Neutrophils were isolated from peripheral blood of women in group A as previously described (21). Polymorphonuclear cells of more than 95% neutrophil purity were used in this experiment. Neutrophils were resuspended in serum free assay medium (RPMI 1640, 1% penicillin-streptomycin, 0.1% BSA) at cell concentration of 1×10^6 cells/ml. Neutrophil chemotaxis was analyzed using transwell inserts (5 μ m pore size; Corning Costar, Corning, NY). The potential chemoattractant, either i) 600 μ l of assay medium (negative control) or ii) 100 nmol *N*-formyl-methionyl-leucyl-phenylalanine diluted in assay medium (positive control) or iii) 600 μ l of medium collected from amnion explants previously treated with either vehicle, 100 ng/ml LPS, 20 mmol sodium propionate, or 100 ng/ml LPS + 20 mmol sodium propionate for 24 h, was added to the bottom chamber of the 24-well plate. Transwell inserts were placed into the wells, and 1×10^5 neutrophils diluted in assay medium (final volume 100 μ l) were

added to the upper compartment. Plates were incubated at 37 C in a 5% CO₂ atmosphere for 1 h. After incubation, excess medium was removed from the upper compartment and transwell filters were placed in methanol for 10 min to fix cells; filters were subsequently stained in Diff-Quick (Baxter Diagnostics, Inc., Deerfield, IL) for 20 min and neutrophils counted microscopically at $\times 400$ magnification (Axiovert 200M; Carl Zeiss). Data are expressed as mean \pm SE of neutrophils per high-power field (10 fields counted) for duplicate filters from five independent experiments.

IL-8 ELISA

Secreted IL-8 was measured in supernatant collected from amnion tissue explants (n = 11 women) treated with vehicle or LPS either alone or in the presence of sodium propionate for 24 h. An in-house ELISA was used, with matched pairs of capture and biotinylated labeled detection antibodies for IL-8 (R&D Systems, Minneapolis, MN). Plates were coated overnight at 4 C with capture antibody (100 μ l/well) followed by 200 μ l/well of dry coat/blocking solution for 1 h at room temperature. After removing the blocking solution, plates were stored at -20 C until analysis. Before use, the plates were washed twice in wash buffer (0.05% Tween 20, 10 mmol/liter Tris, and 0.15 mol NaCl). Recombinant IL-8 standard (R&D Systems) diluted in ELISA buffer (using a concentration range of 1000 to 7.8 pg/ml) and 100 μ l of standard or supernatant sample were added per well.

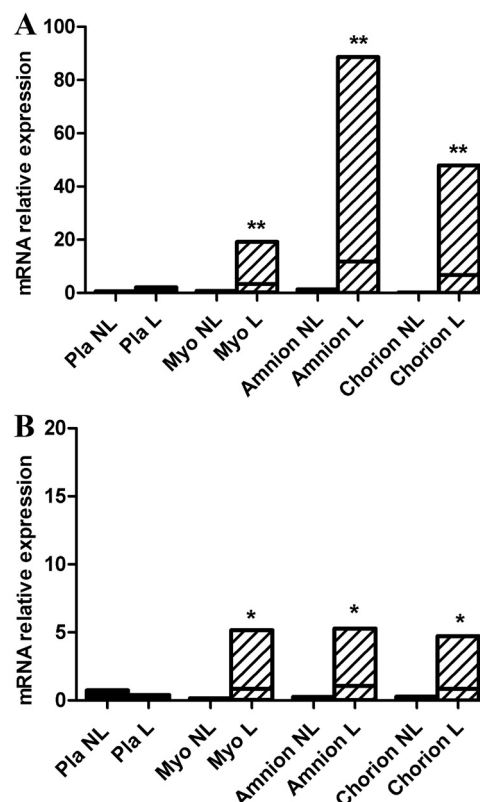


FIG. 1. GPR43 and GPR41 mRNA expression in nonlaboring (NL) and laboring (L) gestational tissues investigated by qRT-PCR. Both GPR43 (A) and GPR41 (B) genes showed significantly higher expression in laboring than in nonlaboring myometrium (Myo), amnion and chorion. No significant differences were found between laboring and nonlaboring placenta (Pla) samples for both receptors. Data are presented as means \pm SE; n = 11 samples. *, $P < 0.05$; **, $P < 0.01$.

Samples were analyzed in duplicate. Plates were incubated overnight at 4°C and then washed before addition of anti-IL-8 detection antibody (R&D Systems) at 60 ng/ml in ELISA buffer. Plates were subsequently washed before addition of streptavidin-peroxidase (250 U/ml; Roche Molecular Biochemicals, Mannheim, Germany) at 1:2000 dilution in ELISA buffer (final concentration 0.125 U/ml) for 20–30 min at room temperature on a rocker. Plates were again washed four times before addition of tetramethylbenzidine substrate. Color change was monitored and stopped using 2 N sulfuric acid. Plates were read on a plate reader at 450 nm within 30 min of quenching. The minimum detection limit of the assay was 15 pg/ml. The interassay and intraassay coefficients of variance were 11 and 9.1% relative SD, respectively.

Statistics

After assessing data distribution, statistical analysis was performed using either (a) one-way ANOVA when comparing three or more data sets or (b) a *t* test when comparing two data sets.

For one-way ANOVA, Friedman's test or non parametric ANOVA with Dunn's posttest were used when data did not assume a Gaussian distribution and ANOVA or repeated-measure ANOVA with Tukey's posttest were applied when the normality test was passed (GraphPad Prism version 5; GraphPad Software, San Diego, CA). All data are presented as mean \pm SE. Significance was assumed whenever $P < 0.05$.

Results

GPR43 and GPR41 mRNA expression in laboring and nonlaboring gestational tissues

GPR43 and GPR41 receptor expression was detected in all tissue samples collected from group A ($n = 11$) and group B ($n = 11$) by qRT-PCR (Fig. 1, A and B). Expression of both receptors was significantly higher ($P < 0.01$ and $P < 0.05$ for GPR43 and GPR41, respectively) in

myometrium, amnion, and chorion but not placenta collected from laboring compared with nonlaboring women. Expression of GPR43 mRNA was highest in laboring amnion compared with other gestational tissues.

GPR43 protein localization in laboring and nonlaboring gestational tissues

GPR43 protein was localized by immunohistochemistry (Fig. 2, A–F). In the nonlaboring myometrium (Fig. 2A), GPR43 protein localized to smooth muscle cells and vascular endothelial cells; in the laboring myometrium (Fig. 2B), the protein additionally localized to the immune cells infiltrating the tissue and inside the blood vessels (1, 3, 6). In the placenta, GPR43 was detected on the syncytiotrophoblast layer and in immune cells in fetal blood vessels and infiltrating the trophoblast (Fig. 2, C and D, respectively). Finally, in amnio-chorion, GPR43 was localized to amniotic epithelium, chorionic trophoblasts, and macrophages within the chorion mesoderm (Fig. 2, E and F). Using current commercially available antibody for GPR41, we have been unable to identify GPR41 staining; thus, localization of GPR41 expression in utero-placental tissues remains to be elucidated.

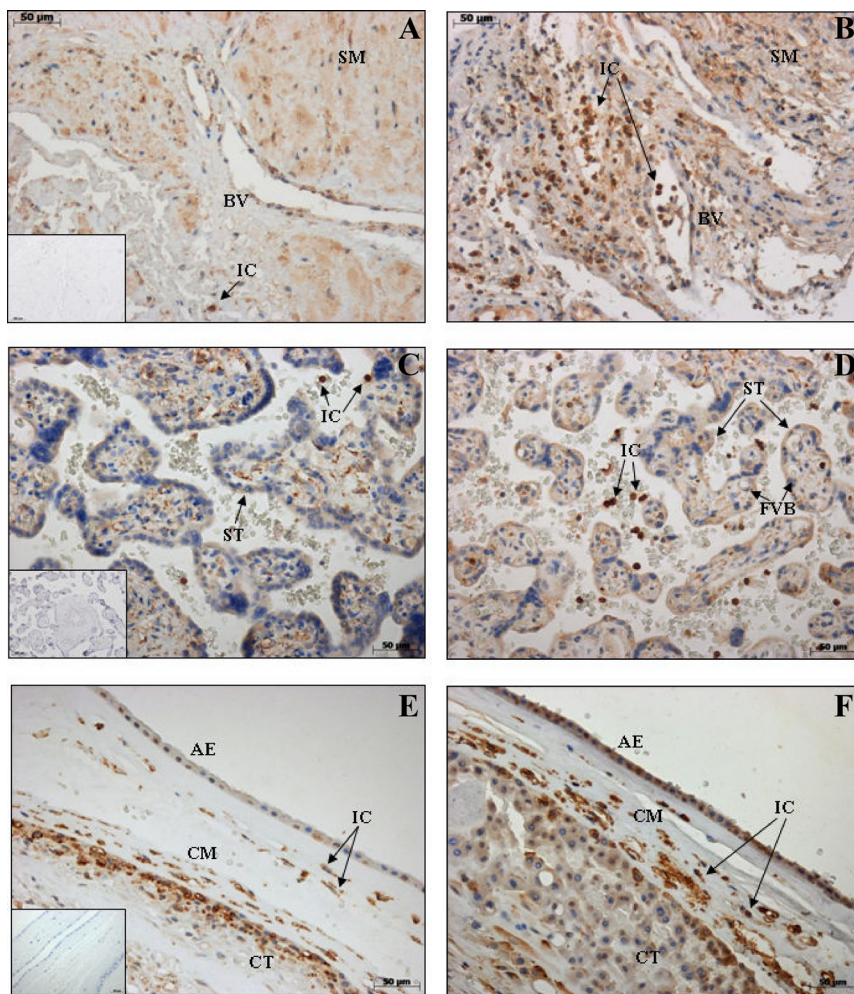


FIG. 2. A–F, Localization of GPR43 protein in term nonlaboring and laboring gestational tissues. Nonlaboring (A) and laboring (B) myometrium, nonlaboring (C) and laboring (D) placenta, and nonlaboring (E) and laboring (F) fetal membranes show a positive staining for GPR43 protein. Scale bar, 50 μ m. Negative controls are shown in inset (scale bar, 100 μ m in the myometrium and placenta and 50 μ m in the fetal membranes). SM, Smooth muscle cells; BV, blood vessel endothelium; IC, immune cells; SC, syncytiotrophoblast layer; FVB, fetal blood vessels; AE, amniotic epithelium; CM, chorion mesoderm; CT, chorion trophoblast.

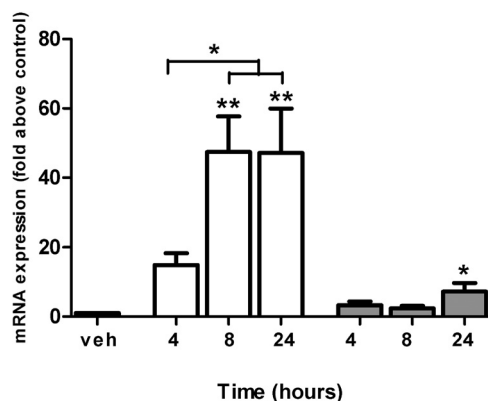


FIG. 3. GPR43 and GPR41 mRNA expression in term nonlaboring amnion explants treated with either vehicle (veh) or 100 ng/ml LPS. LPS induced a significant increase in GPR43 mRNA expression (white bars) at 8 and 24 h compared with vehicle treatment (black bar) or treatment with LPS for 4 h. GPR41 mRNA expression (gray bars) increased significantly at 24 h LPS treatment. Data are presented as means \pm SE; $n = 11$ samples. *, $P < 0.05$; **, $P < 0.01$.

***In vitro* GPR43 and GPR41 regulation by LPS in human amnion explants**

Because GPR43 mRNA levels were highest in the amnion, we subsequently investigated the effect of a mimetic of infection, LPS, on the expression of GPR43 and GPR41 in amnion explant cultures. Term nonlaboring human amnion explants (group A) were treated with either vehicle or 100 ng/ml LPS (Fig. 3). LPS treatment induced a significant increase in GPR43 mRNA expression (measured by qRT-PCR) at 8 h (47.4 ± 10.3 -fold above control, $P < 0.01$) and 24 h (47.2 ± 12.8 -fold above control, $P < 0.01$). LPS induced a modest increase in GPR41 mRNA expression at 24 h of treatment (7.3 ± 2.2 -fold above control, $P < 0.05$).

The SCFA sodium propionate reduces LPS-induced expression of pro-inflammatory cytokines and adhesion molecules

The mRNA expression of proinflammatory cytokines (IL-6, IL-8, COX-2, IL-1 α , IL-1 β) and adhesion molecules (ICAM-1, LFA-1, PECAM-1) in human amnion explants in response to treatment with LPS alone or in the presence of sodium acetate or sodium propionate for 8 and 24 h is presented in Fig. 4. In accordance with previous *in vitro* studies, treatment of fetal membranes with LPS increased the expression of the proinflammatory cytokines (22, 23). Conversely, we found that cotreatment with sodium propionate reduced the LPS-induced expression of IL-6 at 8 and 24 h ($P < 0.05$; Fig. 4A), IL-8 and COX-2 at 24 h ($P < 0.05$ and $P < 0.01$, respectively; Fig. 4, B and C), and IL-1 α at 24 h ($P < 0.01$; Fig. 4D). LPS-induced expression of IL-1 β was not significantly affected by cotreatment with sodium propionate (Fig. 4E), whereas cotreatment with sodium acetate had no significant inhibitory effect on LPS-induced expression of any of the studied pro-inflammatory genes (Fig. 4, A–E). LPS has been

shown previously to increase ICAM-1 mRNA expression in endothelial cells, nongestational tissues (24, 25), and syncytiotrophoblast cells (26). However, the effect of LPS on LFA-1 and PECAM-1 expression in gestational tissues has not been elucidated and studies on nongestational tissues have shown contrasting results (27–29). As expected, treatment with LPS increased expression of ICAM-1 mRNA. Cotreating the amnion explants with LPS and the SCFA, either sodium propionate or sodium acetate, significantly reduced the LPS-induced ICAM-1 mRNA expression at 8 h after treatment with sodium acetate ($P < 0.01$) and at 8 and 24 h after treatment with sodium propionate [$P < 0.01$] (Fig. 4F). Treatment with LPS alone did not alter PECAM-1 mRNA expression; however, cotreatment with LPS and sodium propionate reduced expression of PECAM-1 at 8 and 24 h ($P < 0.01$) compared with LPS treatment alone (Fig. 4G). LFA-1 mRNA expression was not altered by any of the treatments (Fig. 4H).

Sodium propionate reduces LPS-induced neutrophil chemotaxis *in vitro*

We subsequently determined whether the SCFA sodium propionate could affect the LPS effect on neutrophil chemotaxis (30). Chemotaxis of neutrophils from pregnant women was significantly elevated after culture with conditioned medium collected from amnion explants treated with 100 ng/ml LPS compared with conditioned medium collected from amnion explants treated with vehicle control ($P < 0.001$). Conditioned medium from explants cultured with 100 ng/ml LPS and 20 mM sodium propionate reduced neutrophil chemotaxis compared with conditioned medium from explants treated with LPS alone ($P < 0.05$) (Fig. 5A).

Sodium propionate reduces IL-8 protein secretion in amnion explants treated with LPS

IL-8 is a chemokine known to induce chemotaxis of neutrophils (31). Because we found that treatment with sodium propionate reduces LPS-induced IL-8 mRNA expression and neutrophil chemotaxis, IL-8 protein levels were assessed in medium collected from human amnion explants treated with vehicle, LPS, or LPS and sodium propionate for 24 h. IL-8 secretion was elevated in explants treated with LPS compared with vehicle (4.7 ± 1.1 vs. 181.4 ± 30.6 ng/ml for vehicle and LPS treatment, respectively, $P < 0.001$). Cotreatment with LPS and sodium propionate significantly reduced IL-8 secretion compared with explants treated with LPS alone (121.2 ± 19.1 vs. 181.4 ± 30.6 ng/ml, respectively, $P < 0.05$) (Fig. 5B).

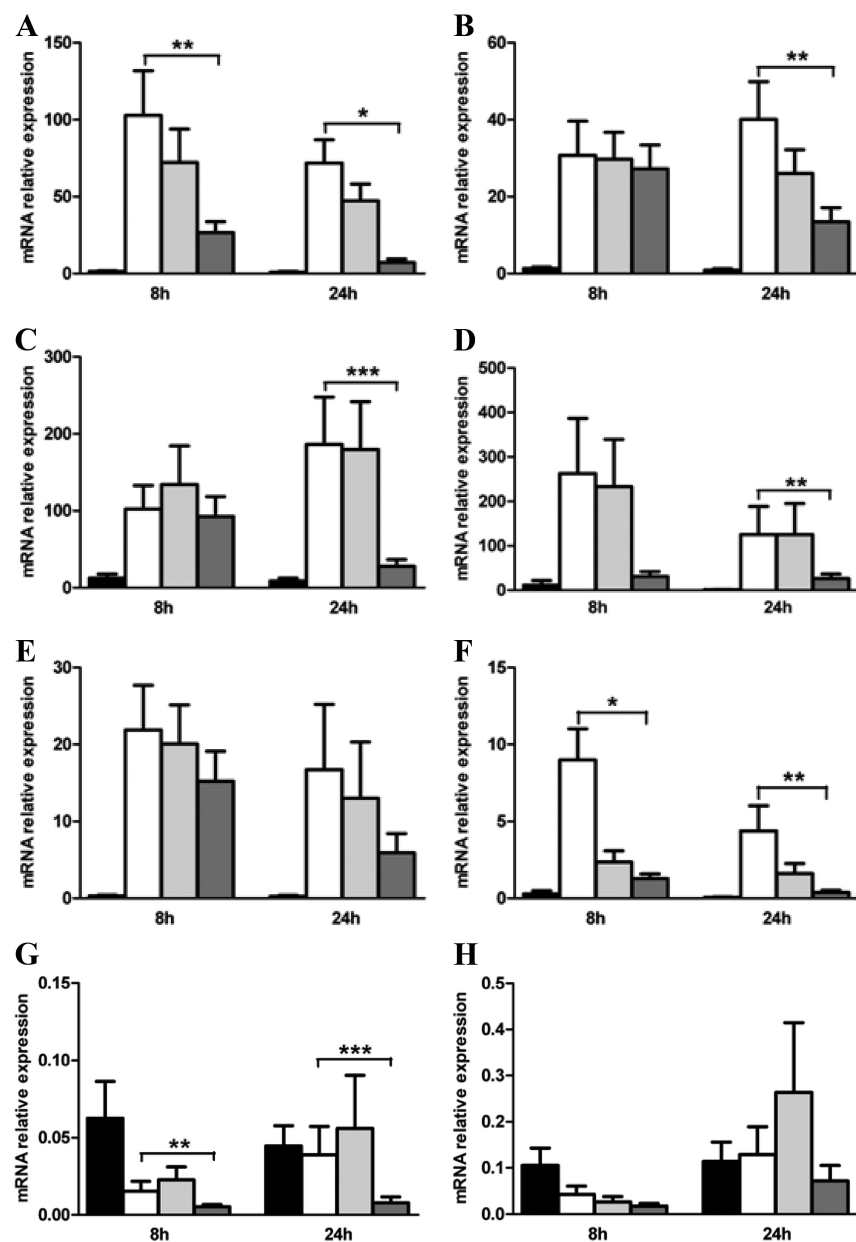


FIG. 4. mRNA expression of proinflammatory cytokines in human term nonlaboring amnion explants after treatment with vehicle (black bars), 100 ng/ml LPS (white bars), 100 ng/ml LPS + 20 mmol sodium acetate (light gray bars), or 100 ng/ml LPS + 20 mmol sodium propionate (dark gray bars). Sodium propionate reduced LPS-induced expression of IL-6 (A), IL-8 (B), COX-2 (C), and IL-1 α (D), but not IL-1 β (E), in a time-dependent manner. Treatment with sodium acetate had no effect on proinflammatory cytokine expression. LPS-induced ICAM-1 mRNA expression (F) was significantly reduced by sodium propionate over the time courses, whereas PECAM-1 (G) expression decreased after LPS + sodium propionate treatment at 24 h, although not affected by LPS treatment at any time course. LPS-induced LFA-1 (H) mRNA expression was not affected by either LPS alone or LPS + SCFA treatment. Data are presented as means \pm SE; $n = 11$ samples. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Data were log transformed to perform statistical analysis.

In vivo GPR43 and GPR41 mRNA expressions in preterm fetal membranes and placenta

To determine whether GPR41 and GPR43 may play a role in the pathophysiology of parturition, expression of GPR43 and GPR41 mRNA was measured by qRT-PCR in fetal membranes and placenta collected from women de-

livering preterm with intact membranes (PTL), pPROM, or pPROM associated with CA (pPROM+CA). Expression of GPR43 (Fig. 6A) was significantly higher in fetal membranes collected from women with pPROM+CA compared with fetal membranes collected from women with PTL or pPROM. Expression of GPR41 (Fig. 6C) was higher in fetal membranes of women with pPROM+CA compared with fetal membranes collected from women with PTL. There was no difference in the expression of GPR43 or GPR41 in placental tissues collected from the three groups of women (Fig. 6, B and D).

Discussion

We have shown, for the first time, expression of the SCFA receptors (GPR43 and GPR41) in the human uterus and related gestational tissues. We have also demonstrated that LPS induces GPR43 (and to a lesser extent GPR41) mRNA expression in human amnion in culture. The GPR41 and GPR43 receptors appear functional, in that SCFA (their natural ligands) inhibit LPS induced pro-inflammatory cytokine expression and neutrophil chemotaxis in amnion explants. Lastly, elevated expression in fetal membranes of women with combined chorioamnionitis and pPROM but not pPROM alone or PTL in the absence of pPROM/CA, suggests a potential role for GPR41 and GPR43 in the pathology of infection induced preterm labor.

The SCFA receptors (GPR43 and GPR41) are well known to be functionally involved in regulating the immune system. Although these receptors can be activated by the same ligands, their expression patterns show different tissue distribution.

GPR43 expression is characterized by high specificity for immune tissues and inflammatory cells (9, 11): with expression mainly restricted to neutrophils, monocytes, and (at lower levels) peripheral blood mononuclear cells. In comparison with GPR43, the GPR41 re-

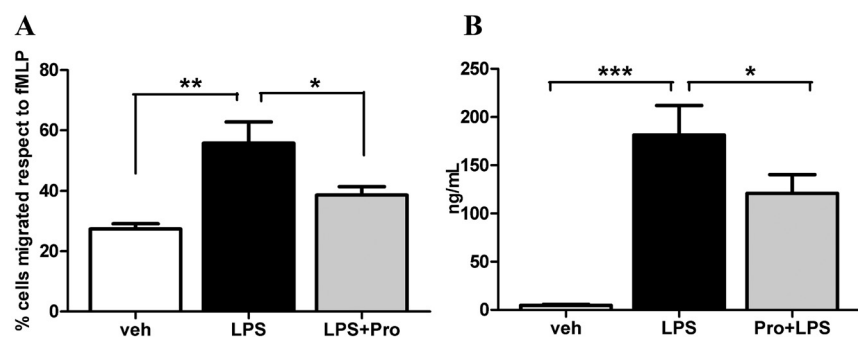


FIG. 5. A, Neutrophil chemotaxis measured by transwell assay in culture medium collected from human amnion explants treated with vehicle (veh), 100 ng/ml LPS, or 100 ng/ml LPS and 20 mmol sodium propionate (Pro) for 24 h. Neutrophil chemotaxis was significantly increased in medium collected from amnion explants treated with LPS compared with vehicle control and was significantly reduced after culture with medium collected from amnion treated with LPS and sodium propionate. Data are presented as percentage of cells that migrated in response to conditioned medium compared with the number of cells that migrated in response to the positive control [*N*-formyl-methionyl-leucyl-phenylalanine (fMLP)]. *, $P < 0.05$; ***, $P < 0.001$. B, IL-8 protein levels in medium collected from human amnion explants treated with either vehicle or LPS \pm sodium propionate for 24 h. IL-8 secretion was reduced after treatment with LPS and sodium propionate compared with LPS treatment alone. Data are presented as means \pm SE. *, $P < 0.05$; ***, $P < 0.001$.

ceptor is more widely expressed in several tissues including adipose tissue, spleen, and lymph node as well as leukocytes (11, 32).

Although we have shown gene expression for both receptors, availability of reagents meant that we were only able to localize protein expression for GPR43 and not GPR41. GPR43 protein was localized to multiple cellular

compartments including infiltrating immune cells. We demonstrated functional effects of GPR41 and GPR43 ligands (the SCFA): again, lack of availability of antagonists meant that we were unable to investigate the differing roles of SCFA via GPR41 and GPR43 in gestational tissues. Evidence attests that GPR43 mediates antiinflammatory activity. GPR43 is involved in neutrophil recruitment during intestinal inflammation (33), and GPR43-deficient mice demonstrate exacerbated or unresolved inflammation in colitis, arthritis, and asthma models, even after SCFA supplementation (19). Both GPR43 and GPR41 mediate additional, nonimmune effects, including adipogenesis and inhibit lipolysis (GPR43) and stimulation of leptin activity (GPR41) (34).

GPR43 knockout mice display loss of acetate mediated reduction in plasma free fatty acid levels (34).

A role for inflammation in physiological and pathological parturition is well recognized (35). In fact, human parturition at term and preterm are associated with a high density of leukocytes and inflammatory cells in the myometrium, cervix, and fetal membranes (3, 36). Labor is also associated with increased pro-inflammatory cytokine production in both gestational tissues (4, 36) and peripheral blood inflammatory cells (7). Proinflammatory cytokines, in turn, stimulate prostaglandin and matrix metalloproteinase release at tissue level that trigger the mechanisms of labor, such as myometrial contractility, rupture of the membranes, and cervical ripening. PTL is widely considered to be triggered by inflammatory processes, either in the presence or absence of infection. Focal infection and inflammation play a major role in the pathogenesis of CA, defined as inflammation of the amniochorionic membranes of the placenta in response to microbial invasion (anaerobic, aerobic, and atypical bacteria) or due to other pathological processes (37). The definition of histological CA is based on microscopic evidence of inflammation of the fetal membranes characterized by infiltration of neutrophils and other immune

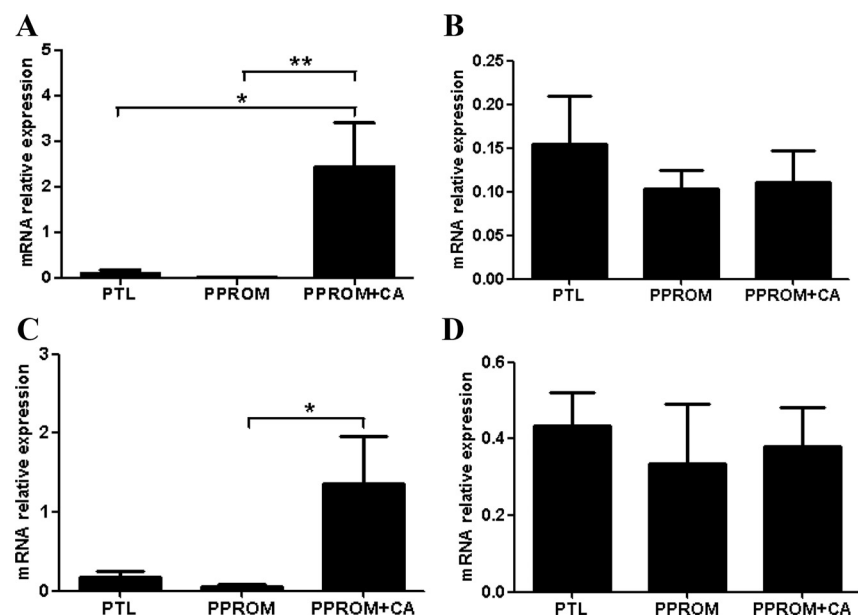


FIG. 6. GPR43 and GPR41 mRNA expression measured by qRT-PCR in fetal membranes and placenta samples collected from women delivering preterm: 1) PTL ($n = 9$), 2) pPROM ($n = 10$), or 3) pPROM + CA ($n = 7$). A, Fetal membranes GPR43 mRNA expression was significantly higher in samples from women with pPROM + CA compared with women with PTL and with pPROM. B, Placental GPR43 mRNA expression was similar among the three groups. C, Expression of GPR41 mRNA in fetal membranes was significantly increased in pPROM + CA samples compared with pPROM group. D, Placental GPR41 expression was similar in the three groups. Data are presented as means \pm SE. *, $P < 0.05$; **, $P < 0.01$.

cells. We demonstrated functional effects of GPR41 and GPR43 ligands (the SCFA): again, lack of availability of antagonists meant that we were unable to investigate the differing roles of SCFA via GPR41 and GPR43 in gestational tissues. Evidence attests that GPR43 mediates antiinflammatory activity. GPR43 is involved in neutrophil recruitment during intestinal inflammation (33), and GPR43-deficient mice demonstrate exacerbated or unresolved inflammation in colitis, arthritis, and asthma models, even after SCFA supplementation (19). Both GPR43 and GPR41 mediate additional, nonimmune effects, including adipogenesis and inhibit lipolysis (GPR43) and stimulation of leptin activity (GPR41) (34).

cells, such as macrophages and T cells. Regardless of the infectious etiology, histological CA challenges the functional integrity of fetal membranes with important pathological consequences (38). Given the role of inflammation in labor, our data demonstrating elevated expression of GPR41 and GPR43 in gestational tissues during both term labor and CA associated preterm labor suggest that increased signaling through these receptors might have functional effects during parturition, dampening down a pro-inflammatory cytokine response and inhibiting neutrophil chemotaxis.

Concerning intracellular pathways that drives SCFA receptor up-regulation in response to inflammatory stimuli, little is known about transcriptional regulation of GPR43 expression. One potential candidate could be the nuclear factor- κ B pathway, which is potently activated by LPS (39, 40). However, further research elucidating the mechanisms governing expression of GPR43 would be worthwhile. Evidence to date suggests that increased ligand/GPR41 or GPR43 receptor interactions occurring at term or infection associated PTL is modulated at the receptor level: although SCFA concentrations are known to increase with anaerobic infections (41), there are no published reports on changes in circulating short chain fatty acid concentrations in term or PTL. It is, however, possible that these receptors can be activated by other endogenous ligands not yet identified. Indeed, Milligan *et al.* (42) have suggested that the low potency of SCFA on GPR41 and GPR43 indicates they may be surrogate rather than endogenous agonist. Synthetic ligands for GPR43 are being developed (43). This raises the possibility of pharmacological activation of this receptor *in vivo*. Such activation could potentially be useful in infection induced PTL to inhibit premature activation of pro-inflammatory events of parturition. Such agents could be of benefit, in combination with antimicrobial therapy, to reduce maternal and fetal inflammation and hence improve outcome in women with preterm labor associated with intrauterine infection/inflammation.

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